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Smear layer removal efficacy of various irrigation solutions with an ultrasonic activation system: an in vitro study

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Purpose: In this *in vitro* study, the smear layer removal efficiency of 17% ethylenediaminetetraacetic acid (EDTA), 10% glycolic acid (GA), and 18% etidronic acid, 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) solutions was evaluated.

Methods: A total of 80 human mandibular premolar teeth were used in the study. The teeth were prepared using the Reciproc R25 rotary instrument system. A total of 10 mL of 2.5% NaOCl irrigation solution was used: 5 mL during the procedure and 5 mL after it. The teeth were divided into four groups (n = 20): group 1, 17% EDTA + passive ultrasonic activation (PUI); group 2, 10% GA+PUI; group 3, 18% HEBP + PUI; and group 4 (control group), distilled water + PUI. Based on the central parts of the coronal, middle, and apical thirds images were taken under a low vacuum scanning electron microscopy at 2000× magnification. The presence of smear layer in the coronal, middle, and apical thirds was evaluated using a five-score evaluation system. Data were analyzed with Kruskal–Wallis and Dunn tests.

Results: No statistically significant difference was found among the groups 1, 2, and 3 in all regions ($p>0.05$).

Conclusion: EDTA, GA, and HEBP irrigation did not affect the smear layer removal by PUI.

Keywords: EDTA, Etidronic acid, Glycolic acid, passive ultrasonic irrigation, Smear layer.

Introduction

The removal of vital and necrotic pulp tissues, microorganisms, and microbial toxins from the root canal system is essential for successful endodontic treatment (1). The shaping of the root canal system, irrigation, and intracanal medicaments play a role in the removal of these remnants. Complex structures such as the isthmus, lateral, and accessory canals cannot be cleaned by conventional cleaning and shaping procedures. To clean these areas, the properties and activation methods of irrigation solutions are gaining great importance (2).

During root canal preparation, a smear layer, which has an organic and inorganic components, is formed (3). In the root canal, the smear layer, limits the effect of irrigants and intracanal medicaments and creates a barrier between root canal obturation materials and dentin tubules, that may cause microleakage (4). Thus, the smear layer must be removed. Because a single agent is insufficient to remove the smear layer, dual irrigation solutions are preferred as the final irrigation. The most recommended combination is ethylenediaminetetraacetic acid (EDTA) and NaOCl (5).

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While the organic part of the smear layer is removed by NaOCl, the inorganic part is removed by EDTA (6).

EDTA, at a concentration of 17%, reacts with the dentin to form calcium chelates. However, as main disadvantages, EDTA does not have antimicrobial properties, creates erosive areas in the dentinal tubules when used for >1 min, limits antimicrobial activity by reacting with NaOCl, and has a limited effect on smear layer removal in the apical third (7,8).

Glycolic acid (GA) is the smallest member of the group of organic acids known as alpha-hydroxy acids. It is used as an organic component in the pharmaceutical industry for polymer formation (9). GA is colorless, odorless, and water-soluble. GA's fast absorption from the skin and mineral surfaces, low pKa value, low molecular weight, and biocompatible organic structure have been the reasons for its preference in dental studies. Recent studies have shown that GA is suitable for enamel–dentin etching and has a less cytotoxic effect on fibroblasts than EDTA (10, 11).

Etidronic acid is defined as 1-hydroxyethylidene-1,1-bisphosphonate (HEBP). These bisphosphonates are highly applied systemically biocompatible chelators in the treatment of bone diseases (12). HEBP, chelating agent, has proteolytic and antimicrobial properties, does not cause a reaction when used with NaOCl, and does not affect the antimicrobial properties of NaOCl (13). A study reported that HEBP effectively removes the smear layer and causes less erosion on the dentin than EDTA (14).

The effectiveness of irrigation solutions depends on the direct contact of the irrigants with the canal walls. Conventional syringe-applied solutions are insufficient to remove debris and the smear layer from the root canal system, especially in the apical region. Various irrigation techniques and devices have been designed to address these problems and increase the flow and distribution of irrigants in root canals (5). In principle, the passive ultrasonic activation (PUI) technique involves transmitting acoustic energy to the fluid in the root canal of a vibrating file. The energy is transmitted by ultrasonic waves, and this energy causes the formation of pressurized steam with an acoustic current in the solution. PUI provides flow of irrigants with hard-to-reach areas and supports debris and smear layer removal from the canal walls (15).

To the best of our knowledge, no study has compared these three chelating agents for smear layer removal. Thus, this study aimed to compare the smear layer removal in the coronal, middle, and apical thirds by 17% EDTA, 10% GA, and 18% HEBP solutions activated by PUI in root canals. The null hypothesis of this study was that no significant difference is found between chelating agents in terms of smear layer removal efficiency.

Materials and Methods

This study was approved by the Dicle University Faculty of Dentistry Ethics Committee with the decision dated May 26, 2021, and numbered as 2021/35 andwas carried out according to the principles in the Declaration of Helsinki.The power of the study was calculated with 95% confidence (1-α), 95.2% test power (1-β), and f = 1.559 test power using with a software (G*Power 3.1, Heinrich Heine University, Dusseldorf, Germany). The calculation indicated that the sample size for each group should be a minimum of 13 teeth (16). Inclusion criteria were that: single root, single canal, and single apical foramen extracted for orthodontic or periodontal reasons. Exclusion criteria were: evidence of root canal calcification, apical resorption, immature root apices, root perforation or fracture.

This study used 80 mandibular premolar teeth with single roots, single canals, and apical development; without caries, fracture, or crack lines; and that were extracted for orthodontic and periodontal reasons. The debris, tartar, and soft tissues on the teeth were removed, and the teeth were kept in distilled water at room temperature. To obtain a standard root length, the crowns of the teeth were to obtain 15-mm root length using a diamond bur under water cooling. Teeth with an apical diameter not wider than #15 K-file were selected for this study. The working length of the remaining roots was determined under a stereomicroscope (15×), from the apical foramen, 1 mm after the tip of the #15 K-file (VDW, Munich, Germany) was visible.

The apex of each tooth was covered with pink wax and embedded in polysiloxane elastomer impression material (Zetaplus, Zhermack, Badia Polesine, Italy) to simulate the anatomical situation in which the periapical tissues protected it. Root canals were prepared using the Reciproc R25 (VDW, Munich, Germany) Ni–Ti rotary file system. Irrigation was performed with 10 mL of 2.5% NaOCl during and after the mechanical preparation, and a 30-G syringe needle (NaviTip, Ultradent Product Inc., South Jordan, USA) was used. During irrigation, the needle tip was moved back and forth at a location 1-2 mm behind the working length. Then, each canal was washed with 5 mL of distilled water and dried with paper cones. Before the final irrigation protocol, the teeth were randomly divided into three experimental groups and one control group (n = 20). Root canal preparation and irrigation activation were performed by a single operator (F. Ç.).

Group 1: Five mL 17% EDTA (Saver, Prime Dental Products PVT Ltd., Maharashtra, India) was applied to the canals. After the canal cavity was filled with EDTA, a gold-type ultrasonic endodontic tip (Eighteeth, Changzhou, China) was attached to the PUI device (Eighteeth, Changzhou, China), and the device was activated for 1

min at maximum power. The ultrasonic tip was carefully placed 2 mm shorter than the apex, without touching the canal walls. The canals were washed with 5 mL of distilled water and dried with paper cones.

Group 2: Five mL 10% GA (Doğal Eczane İlaç Koz. Gıda. San. Tic. Ltd. Şti, Izmir, Turkey) was applied, as described in group 1.

Group 3: Five mL 18% HEBP (Akbel Kimya San. Tic. Ltd. Şti, Bursa, Turkey) was applied, as described in group 1.

Group 4 (control group): Distilled water (5 mL) was administered, as described in group 1.

Parallel grooves were prepared along the buccal and lingual surfaces of the teeth, whose chemomechanical preparation was completed, with the help of a thin flame-tipped bur attached to a high-speed air turbine, under water cooling, and without touching the inner surface. The roots were then divided into two parts along the longitudinal axis, and only one-half of each root was used for scanning electron microscopy (SEM) (Quanta FEG 250; FEI Ltd., Brno, Czech Republic). Images obtained using the SEM device were taken from the coronal, middle, and apical thirds of the root canals of the teeth at 2000× magnification and under 20,000 kV. Images were carefully taken from the central parts of each region. The images were then evaluated according to Hülsmann's classification in terms of the presence of the smear layer and whether the dentinal tubules were open (17).

- Score 1: No smear layer; dentinal tubules are open.
- Score 2: Small amount of smear layer; some dentinal tubules are open.
- Score 3: Homogeneous smear layer covering the root canal wall; only a few dentinal tubules are open.
- Score 4: Homogeneous smear layer covering the root canal wall; no dentinal tubules were open.
- Score 5: Heavy, inhomogeneous smear layer covering the entire root canal wall; no open dentinal tubules.

Two blind independent operators evaluated the SEM images and scored separately (S.K, F. Ç).

Statistical Analysis

Data were analyzed with IBM SPSS Statistics for Windows version 23 (IBM Corp., Armonk, NY, USA). Conformity to a normal distribution was evaluated with the Kolmogorov–Smirnov test. The Kruskal-Wallis H test was used to compare non-normally distributed data, and multiple comparisons were analyzed with Dunn's test. The results were presented as mean ± standard deviation and median (minimum-maximum) for quantitative data and frequency (%) for categorical variables. Cohen's kappa statistics were used to calculate interobserver agreement. The significance level was taken as $p<0.05$.

Results

The results of the Kappa test showed high agreement between the observers (kappa value = 0.866). According to the results of the statistical analysis, a significant difference was found between the experimental groups and the control group ($p<0.05$) (Table 1). Although the solutions removed the smear layer in the coronal third and middle third parts of the teeth more effectively, less smear layer was removed in the apical region. While the control group had the lowest efficiency in the removal of smear layer, no significant difference was found among EDTA, GA, and HEBP ($p> 0.05$) (Table 1 and Fig. 1).

Discussion

The presence of a smear layer in root canal systems causes the microorganisms in its content to reproduce easily, penetrate easily into the dentinal tubules, and reduce the effectiveness of the applied irrigation solutions (18). No single solution has all the properties needed to remove the smear layer. The combination of NaOCl and EDTA is the most routinely used irrigation protocol in clinics. The ef-

Table 1. Statistical test results showing smear layer score after using irrigation solutions and activation with PUI

	EDTA	GA	HEBP	DW
Coronal	1 (1–3)^{a,1}	1 (1–2)^{a,1}	1.5 (1–2)^{a,1}	4 (3–5)^{b,2}
Middle	2 (1–3)^{a,1}	1 (1–2)^{a,1}	2.5 (1–3)^{a,1}	5 (4–5)^{b,2}
Apical	3 (2–4)^{c,3}	2.5 (1–4)^{c,3}	3 (1–4)^{c,3}	5 (4–5)^{b,2}

Kruskal–Wallis H test; ^{a,b,c}: No difference was observed between groups with the same letter in each line ($p>0.05$); ^{1,2,3}: No difference was observed between groups with the same numbers in each column ($p>0.05$)

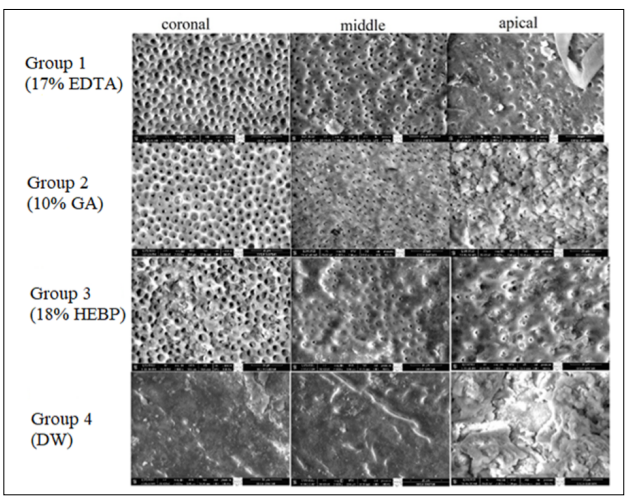

Fig. 1. Representative scanning electron micrographs of the three canal thirds (coronal, middle, and apical) of the group tested (×2000)

fectiveness of using 17% EDTA, 10% GA, and 18% HEBP together with the PUI technique in removing the smear layer was compared in our study.

In previous studies comparing the smear layer removal efficacies of different final activation procedures, PUI was reported to be more effective than other sonic and conventional irrigation methods (19,20). The success of PUI depends on the high speed and volume of the irrigant solution in the canal and therefore better penetration of the irrigant into the dentinal tubules (21). Therefore, PUI was preferred as the activation method in our study.

In dentin erosion and smear layer studies, dentin sections are generally examined under 1000x magnification (20,22). In our study, we preferred 2000× magnification (23), where we could see the dentinal tubules and the peritubular dentin at the same time.

In the present study, no statistically significant difference was found between the smear layer removal efficiency of the EDTA, GA, and HEBP groups ($p > 0.05$). Thus, the null hypothesis was confirmed. Although no statistically significant difference was found between the solutions, the order of success in removing the smear layer was determined as GA > EDTA > HEBP.

Dal Bello et al. evaluated the smear layer removal efficiency of 5%–10% to 17% GA, 17% EDTA, and 10% citric acid solutions and found that GA showed similar properties to EDTA and citric acid in removing the smear layer without any significant difference between the concentrations used (11). In general, our study supports the results of this research.

Kuruvilla et al. evaluated the smear layer removal efficiency of 17% EDTA, 18% HEBP, and 7% maleic acid solutions. In a previous study, maleic acid showed better smear layer removal than EDTA and HEBP; however, between EDTA and HEBP, there was no significant difference observed (24). Mankeliya et al. evaluated the smear layer removal efficiencies of 17% EDTA, 18% HEBP, 10% citric acid, and 7% maleic acid irrigation solutions in the apical third of the root canal. They showed that 7% maleic acid removed the smear layer better than other solutions. According to their study, 10% citric acid was found to be more efficient than EDTA and etidronic acid (25). Both these studies support our findings in terms of EDTA and HEBP.

De-deus et al. examined the time-based smear layer removal efficiency of HEBP and EDTA and reported that EDTA completely removed the smear layer within 1 min, whereas HEBP achieved this effect after 5 min (26). This situation demonstrates a difference from the results of our study. This difference between the results may have been due to the use of the PUI system in our study.

The higher efficiency of the GA solution compared to EDTA and HEBP may be attributed to its low surface tension and small particles in its structure. Thus, it may have removed the smear layer by providing better penetration into the dentin surface. Despite being a weak chelating agent, HEBP has a smear layer removal efficiency that is equal to that of GA and EDTA, demonstrating its importance for endodontic use. One of the expected properties of irrigation agents is that while effectively removing the smear layer, they should not create erosion areas in the dentin tissue. HEBP, a weak chelator, may be advantageous in this respect. Furthermore, HEBP can be used as a chelating agent in clinical use because it does not react with NaOCl and its combined use does not reduce the tissue-dissolving and antibacterial activity of NaOCl.

In the group comparison, EDTA and GA solutions removed the smear layer more effectively in the coronal and middle third regions than in the apical region. No statistically significant difference was observed between the coronal and middle third regions. In the HEBP solution, the smear layer was significantly removed in the coronal third region relative to the apical third. Although a passive ultrasonic system was used in our study, the smear layer in the apical third could not be completely removed, as in many other studies. The lower removal rate of the smear layer in the apical third compared with other regions may be due to factors such as a narrower apical third, dentin tubule structure, contact time of the solutions, and less depth of penetration. In addition, the vapor lock formed as a result of the compression of air bubbles in the apical third during irrigation reduces the effect of irrigation solutions in this area (27).

Due to the *in vitro* nature of this study it is difficult to simulate clinical usage of the solutions. The presence of tissue residues, such as blood, in in vivo studies, variable temperature, application of various activation devices, and sclerotic changes in the dentin, root canal length, diameter, and curvature may affect the structure of chelating agents used during root canal preparation.

Conclusion

Further research is required and more *in vivo - in vitro* studies are needed more accurately to convey the benefits and results of such irrigation agents to clinicians and to evaluate the structural properties of these agents in detail.

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